Agilent ChemStation for LC Systems

Getting Started

Agilent Technologies

Notices

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In This Guide...

This Getting Started guide provides detailed instructions for the first steps with your Agilent LC ChemStation. If you follow the sections through in sequence, you will cover all the basic operations of the ChemStation sufficient to start running your own samples. You can also use the individual sections to learn about a specific task, or as reminders when you need to do a specific task.

1 Equilibrating the System

This exercise leads you through the first steps in operating your Agilent 1100 system with the ChemStation.

2 Setting Up a Method to Run a Checkout Sample

This exercise tells you how to set up the system to run a single sample and obtain a chromatogram.

3 Integrating the Signal

When you have obtained a good chromatogram, you use these instructions to load the signal and integrate it.

4 Setting Up Calibrations

In this exercise, you use a set of demonstration data files to set up different types of calibrations.

5 Automating Analyses

This exercise guides you through the process of setting up a Sequence to automate your analyses.

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1
Equilibrating the System

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This exercise guides you through the process of equilibrating your Agilent 1100 LC system.

Before You Start

NOTE These instructions describe the equilibration procedure for the Agilent Isocratic Sample, part number 01080-68702. If you intend to use a different sample, adjust the conditions accordingly.

Before you start this exercise, ensure that:

- your Agilent 1100 modules are correctly connected and set up. For details, refer to the hardware manuals that came with your system.
- a suitable column is installed. For the Agilent Isocratic Sample, we recommend a Sorbax Eclipse XDB C-8, 150 mm x 4.6 mm, 5 µm, part number 993967-906.
- all the modules are switched on.
- the ChemStation is correctly configured. Refer to the on-line help for the Configuration Editor and the ChemStation.
- the solvent bottles are full (water in channel A, acetonitrile in channel B).

Configuring the User Interface

The Method and Run Control view of the ChemStation (Figure 1) allows you to display and control the instrument and run parameters.

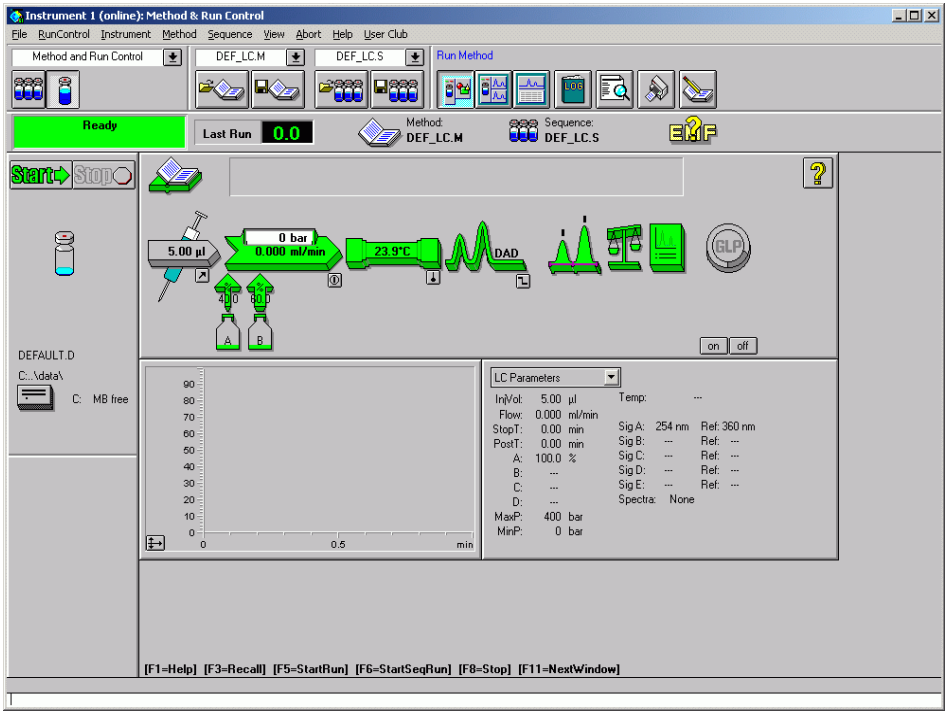


Figure 1 The Method and Run Control view of the ChemStation

1 If necessary, switch to Method and Run Control view:

View > Method and Run Control (see Figure 2 on page 10)

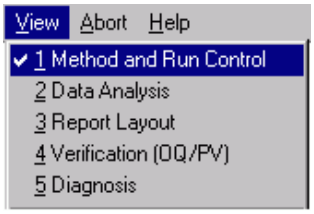


Figure 2 Switching to Method and Run Control view

2 Click in the Run Method toolbar.

3 If necessary, switch to full menus:

View > Full Menu

NOTE This item in the View menu switches between Full Menu and Short Menu, depending on the current state of the menus.

4 Display the Sampling Diagram if it is not already displayed:

View > Sampling Diagram

5 Display the System Diagram if it is not already displayed:

View > System Diagram

Loading the Default Method

1 Display the Load Method dialog box:

File > Load > Method

2 Select **def_lc.m** from the list of methods.

3 Click **OK** to close the dialog box and load the method.

NOTE The name of the active (loaded) method is displayed in the middle combo box at the top of the main toolbar.

Configuring the Online Plot

1 Display the Signal Window:

View > Online Signals > Signal Window 1

2 Click **Change** in the Online Plot window to display the **Edit Signal Plot** dialog box (see Figure 3):

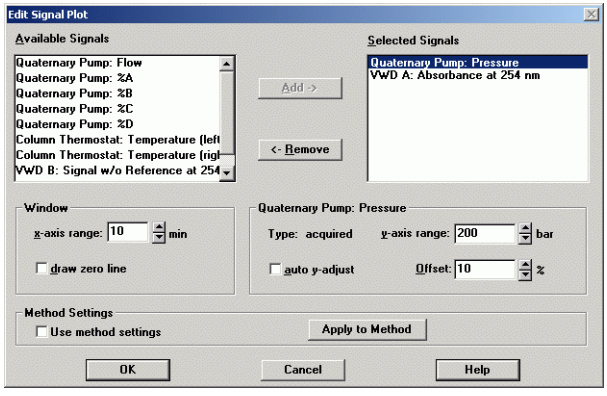


Figure 3 The Edit Signal Plot dialog box

3 From the Available Signals panel, select **DAD 1A**, **MWD 1A** or **VWD A**, depending on the detector you have configured, and click **Add**.

4 From the Available Signals panel, select **Binary/Quaternary Pump Pressure**, depending on the pump you have configured, and click **Add**.

5 In the Selected Signals panel, select pump pressure.

   a In the **Window** group, set the **x-axis range** to `10 min`.

   b In the **Pump Pressure** group, set the **Range** to `200 bar`.

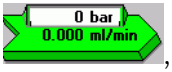
6 In the **Selected Signals** panel, select the detector signal.

   a Set the **y-axis range** to `1000 mAU`

7 Click **OK**.

Purging the Pump

1 Manually open the purge valve on the pump. For details, refer to the hardware manual that came with your pump.

2 In the System Diagram, click on the pump, 

, and select **Set up Pump** from the menu.

3 In the **Control** group. set the **Flow** to `5 ml/min`.

4 In the **Solvents** group, set `50% B` (for binary and quaternary pumps).

5 Click **OK** to close the dialog box.

6 In the System Diagram, click

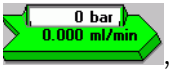
, and leave the pump purging for 10 minutes.

NOTE If you cannot see the On and Off buttons in the System Diagram, move the Online Plot window so that they are visible.

7 After 10 minutes, stop the purging by clicking

.

Setting the Equilibration Conditions

1 In the System Diagram, click on the pump, 

, and select **Set up Pump** from the menu.

   a In the **Control** group. set the **Flow** to `1 ml/min`.

   b In the **Solvents** group, set `80% B` (for binary and quaternary pumps).

NOTE If you intend to use a sample other than the Agilent Isocratic Sample, part number 01080-68702, adjust the parameters for the initial conditions of the analysis.

   c Click **OK** to close the dialog box.

2 After 5 minutes, check that there is pump pressure shown on the online plot.

3 Watch the online plot; when the baselines have stabilized, leave the system pumping for at least 15 minutes to equilibrate.

2 Setting Up a Method to Run a Checkout Sample

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This exercise shows you how to set up a method to acquire data from a standard sample.

Before You Start

NOTE These instructions describe the setup of a method to run the Agilent Isocratic Sample, part number 01080-68702. If you intend to use a different sample, adjust the conditions accordingly.

Before you start this exercise, ensure that:

- a suitable column is installed. For the Agilent Isocratic Sample, we recommend a Sorbax Eclipse XDB C-8, 150 mm x 4.6 mm, 5 µm, part number 993967-906.
- your system is purged and equilibrated, see Chapter 1, “Equilibrating the System”.
- the solvent bottles are full (water in channel A, acetonitrile in channel B).
- you have prepared a sample in a 2 ml vial, sealed with a septum cap.
- the default method, def_lc.m is loaded.

Setting Up the Injector

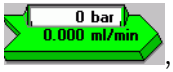
1 In the System Diagram, click on the injector,

, and select **Set up Injector** from the menu.

   a In the **Injection** group, select **Standard Injection** and set the **Injection Volume** to `5.0 µl`.

   b Click **OK** to close the Set up Injector dialog box.

Setting Up the Pump

1 In the System Diagram, click on the pump, 

, and select **Set up Pump** from the menu.

2 In the **Control** group, set the **Flow** to `1 ml/min`.

3 Set the **Stoptime** to `6 min`.

4 Set the **Posttime** to `2 min`.

5 In the **Solvents** group, set `80% B` (for binary and quaternary pumps).

6 In the **Timetable**, click **Append**.

7 Set the **Time** to `2.0 min` and the `%B to 80`.

8 Click **Append** again and set the **Time** to `6 min` and the `%B to 100`.

NOTE If you intend to use a sample other than the Agilent Isocratic Sample, part number 01080-68702, adjust the parameters for the initial conditions of the analysis.

9 Click **OK** to close the dialog box.

10 In the System Diagram, click on the solvent bottles,

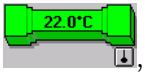
, and select **Solvent Bottles Filling** from the menu.

11 Enter the actual volumes of solvent in A and B.

12 Ensure that the *Prevent analysis if level falls below* and *Turn pump off if running out of solvent* are checked.

13 Click **OK** to close the dialog box.

Setting Up the Column Thermostat

1 In the System Diagram, click on the column, 

, and select **Column Thermostat Method** from the menu.

NOTE If a column-switching valve is installed, ensure that the valve position is set to use the appropriate column.

2 Set the **Temperature** to 25°C.

3 Click **OK** to close the dialog box.

Setting Up the Detector

DAD and MWD

1 In the System Diagram, click on the detector, , and select **Set up DAD Signals** or **Set up MWD Signals** from the menu.

2 In the **Signals** group, select to **Store** wavelengths A and B.

3 In the A row, set **Sample** to `205 nm`, **Bw** to `10 nm`, **Reference** to `400 nm`, **Bw** to `80 nm`.

4 In the B row, set **Sample** to `280 nm`, **Bw** to `10 nm`, **Reference** to `400 nm`, **Bw** to `80 nm`.

5 In the **Required Lamps** group, select both **UV** and **Vis**.

6 In the **Peakwidth (Responsetime)** group, display the drop-down list and select **>0.1 min (2 s)**.

7 In the **Slit** group, display the drop-down list and select **4 nm**.

8 In the **Autobalance** group, select **Prerun**.

9 Click **OK** to close the dialog box.

VWD

1 In the System Diagram, click on the detector, , and select **Set up VWD Signals** from the menu.

2 In the Signals group, set the **Wavelength** to `254 nm`.

3 In the **Peakwidth (Responsetime)** group, display the drop-down list and select **>0.1 min (2 s)**.

4 Click **OK** to close the dialog box.

Saving the Method

1 Display the Save Method as dialog box:

File > Save As > Method

2 In the **Name** field, enter the name `testmeth` and click **OK** to close the dialog box.

3 In the Save Method dialog box, enter a comment (for example, `Method for Checkout Sample`) in the field and click **OK** to close the dialog box.

Running the Method

1 Place the sample vial in position 11 in the autosampler tray.

NOTE If you are using a well-plate sampler, use an appropriate position, for example, 1A1. Refer to the on-line help for full details.

2 Click the **Single Sample** button, , in the toolbar.

3 Display the Sample Info dialog box:

RunControl > Sample Info

4 Enter your name in the `Operator Name` field.

5 In the **Data File** group,

a Select **Prefix/Counter**

b Enter the **Subdirectory** `test` and press **Enter**
If the subdirectory does not exist, a warning message is displayed.

6 In the **Sample Parameters** group,

a Enter `11` in the **Location** field.

b In the **Sample Name field**, enter `Test Sample`.

c Enter a comment for the test sample in the **Comment** field.

7 Click **OK** to close the dialog box.

8 In the Sampling Diagram, click

.

NOTE You can also start the method by clicking **Run Method** in the Sample Info dialog box or by pressing the F5 key on your keyboard.

9 When the run is complete, click

in the System Diagram to turn off the instrument. Click **Yes** to confirm the shut-down.

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Integrating the Signal

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This exercise guides you through the process of integrating a signal.

Before You Start

This exercise uses the data file acquired in Chapter 2, “Setting Up a Method to Run a Checkout Sample”. However, if you wish, you can use the demonstration data file *DEMODAD.D* from the *CHEM32\n\DATA\DEMO* folder (where n is the instrument number).

Integrating a signal

1 If necessary, switch to Data Analysis view:

View > Data Analysis (see Figure 4)

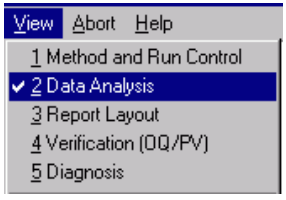

Figure 4 Switching to Data Analysis view

2 In the Data Analysis toolbar, click to switch to the integration workspace.

3 Load the data file acquired in the exercise Chapter 2, “Setting Up a Method to Run a Checkout Sample”:

File > Load Signal

4 In the Load Signal dialog box, switch to the **TEST** folder and select the file.

5 In the Integration/Report toolbar, click to switch to the integration events table.

6 Above the integration events table, click the down arrow to display the list of available signals and select the **VWD1 A** signal, **DAD1 A** signal or **MWD1 A** signal, depending on the detector you used to acquire the data.

For more information about the available signals, see the on-line help.

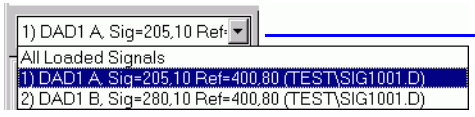
Signal selection down arrow
1) DAD1 A, Sig=205,10 Ref
All Loaded Signals
1) DAD1 A, Sig=205,10 Ref=400,80 (TEST\SIG1001.D)
2) DAD1 B, Sig=280,10 Ref=400,80 (TEST\SIG1001.D)

Figure 5 Selecting the signal

Changing the Initial Events

1 Examine the integration results table below the signal window to gather information about the areas of the integrated peaks.

2 Determine the area of the smallest peak that you want to be integrated.

3 In the integration events table, click in the **Value** column of the **Area Reject** event, and set the value to just below the area of the smallest peak that you want to integrate.

4 In the Integration/Report toolbar, click to reintegrate the signal.

Zooming in to a selected area of the signal

You can check the integration baselines by magnifying a selected part of the signal.

1 In the toolbar, click to switch to the zoom cursor.

2 In the signal window, position the crosshairs of the cursor at the bottom left of the area that you want to magnify (for example, below the signal baseline to the left of the smallest peak).

3 Click the left mouse button, and, keeping the left mouse button pressed, move the crosshairs to the upper right corner of your selected area.

4 Release the mouse button to display the selected area in the signal window.

5 In the toolbar, click to zoom out to the original magnification.

NOTE You can zoom in multiple times to focus on a specific part of the signal. Each time you click the zoom out tool, the magnification returns to the previous setting.

Setting Timed Events

1 In the toolbar, click the down arrow to display the list of integration events (see Figure 6).

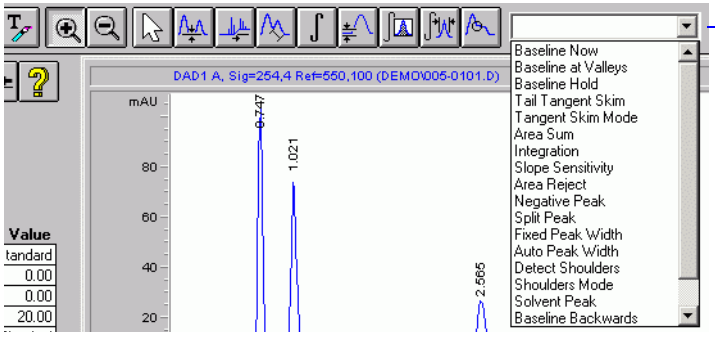

Figure 6 Displaying the list of integration events

2 Select **Integration** from the list.

3 In the signal window, place the cursor to the left of the solvent peak and click the left mouse button.

A line with the event Integration, the value **OFF** and the time selected by the cursor position is appended to the integration events table.

4 Move the cursor to a new position to the right of the solvent peak and click the left mouse button.

An Integration event with the value **ON** and the time selected by the new cursor position is added to the integration events table.

5 In the Integration/Report toolbar, click to reintegrate the signal.

NOTE The solvent peak is no longer integrated, and does not appear in the integration results table

For full details of the available integration events, refer to the on-line help.

6 In the integration workspace toolbar, click to save the modified integration events table and close the integration workspace.

7 In the toolbar, click to display the Save Method dialog box.

8 In the Save Method dialog box, enter a comment (for example, `Modified integration events`) in the field and click **OK** to close the dialog box.

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Setting Up Calibrations

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This exercise guides you through the process of setting up a calibration.

Before You Start

This exercise uses demonstration data files from the folder *CHEM32\n\DATA\DEMO* (where n is the instrument number).

Setting Up a Single-level ESTD Calibration

1 If necessary, switch to Data Analysis view:

View > Data Analysis (see Figure 7)

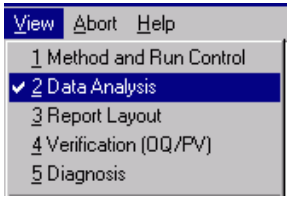

Figure 7 Switching to Data Analysis view

2 In the Data Analysis toolbar, click to switch to the calibration workspace.

3 Load *Signal A* of the first data file, *005-0101.D*:

a **File > Load Signal**

b In the Load Signal dialog box, navigate to the folder *CHEM32\n\DATA\DEMO*, where *n* is the instrument number.

c Select the data file *005-0101.D*.

d Click the [Full >>] button to display the signal information (see Figure 8 on page 32).

e Select the first signal, *DAD1 A* and click **OK**.

f Ensure that **Integrate after load** is checked.

This is a calibration sample containing 100 ng of each component.

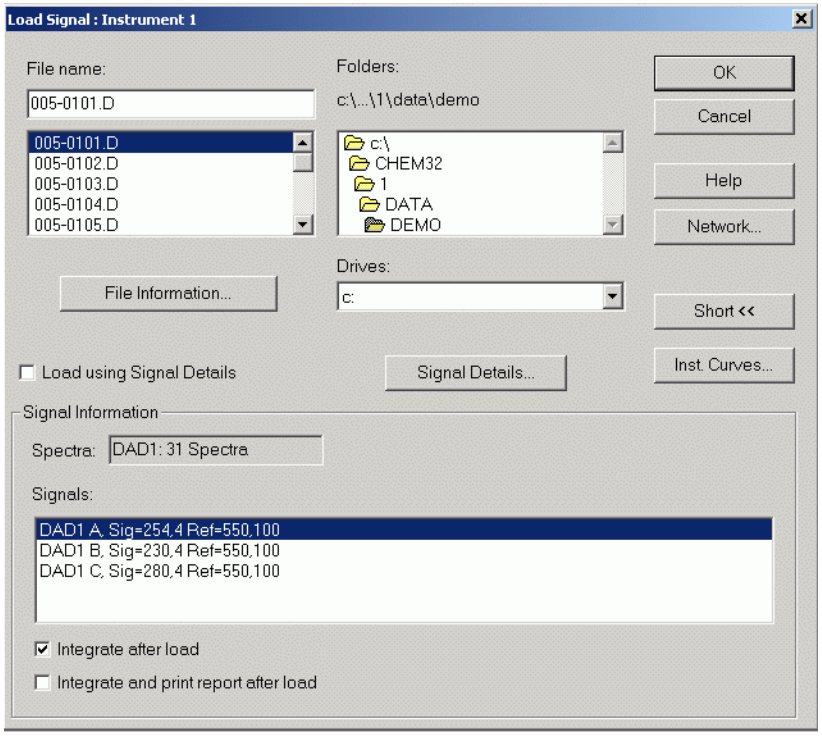

Figure 8 The full Load Signal dialog box

4 Display the Calibrate dialog box (see Figure 9 on page 33):

Calibration > New Calibration Table

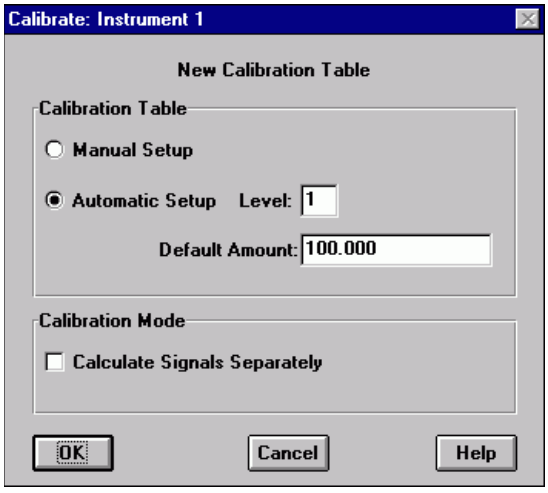

Figure 9 The Calibrate dialog box for a new calibration table

5 In the **Calibration Table** group:

   a Select the **Automatic Setup** option.

   b Set the **Level** to `1`.

   c Set the **Default Amount** to `100`.

6 Click **OK** to close the dialog box and set up the calibration table.

7 In the Compound column of the calibration table, enter compound names for the four compounds (for example, `Compound 1`, `Compound 2`, `Compound 3` and `Compound 4`).

8 Click **OK** to close the calibration table.

Quantifying an Unknown

1 Display the Specify Report dialog box:

Report > Specify Report

2 In the **Destination** group, select **Printer** and **Screen.**

3 In the **Quantitative Results** group:

a Click the down arrow of the **Calculate** combo box and select **ESTD** from the list.

b Ensure that **Based On** is set to **Area**, and **Sorted By** is set to **Signal**.

c Click **OK** to close the dialog box.

4 In the **Style** group, ensure that **Add Chromatogram Output** is selected.

5 Click **OK** to close the **Specify Report** dialog box.

6 Save the method as `testcal.m`:

File > Save As > Method

7 Load the signal *DAD1 A* of the data file *005-0102.D* from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

8 Print a quantitative report to the screen and the printer:

Report > Print Report

Adding Second and Third Levels to the ESTD Calibration

1 Load the signal DAD1 A of the data file 006-0201.D from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

This is a calibration sample containing 200 ng of each component.

2 Display the Calibrate dialog box to add a new level (see Figure 10):

Calibration > Add Level

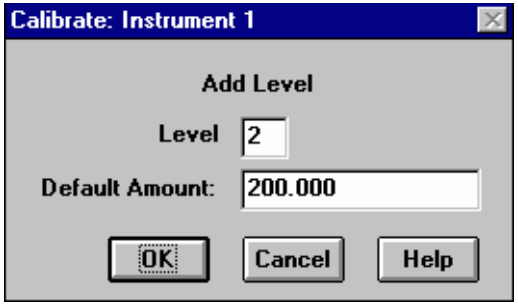

Figure 10 The Calibrate dialog box for adding a new level

3 Ensure that the **Level** is set to 2.

4 Enter `200` in the **Default Amount** field.

NOTE

The calibration table is automatically updated with a new level for each compound.

5 Load the signal *DAD1 A* of the data file *007-0301.D* from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

This is a calibration sample containing 300 ng of each component.

6 Display the Calibrate dialog box to add another level (see Figure 10):

Calibration > Add Level

7 Ensure that the **Level** is set to 3.

8 Enter 300 in the **Default Amount** field.

9 Save the method

File > Save > Method

Quantifying an unknown

1 Load the signal *DAD1 A* of the data file *005-0102.D* from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

2 Print a quantitative report to the screen and the printer:

Report > Print Report

Changing the calibration curve type

1 Display the Calibration Settings dialog box (see Figure 11):

Calibration > Calibration Settings

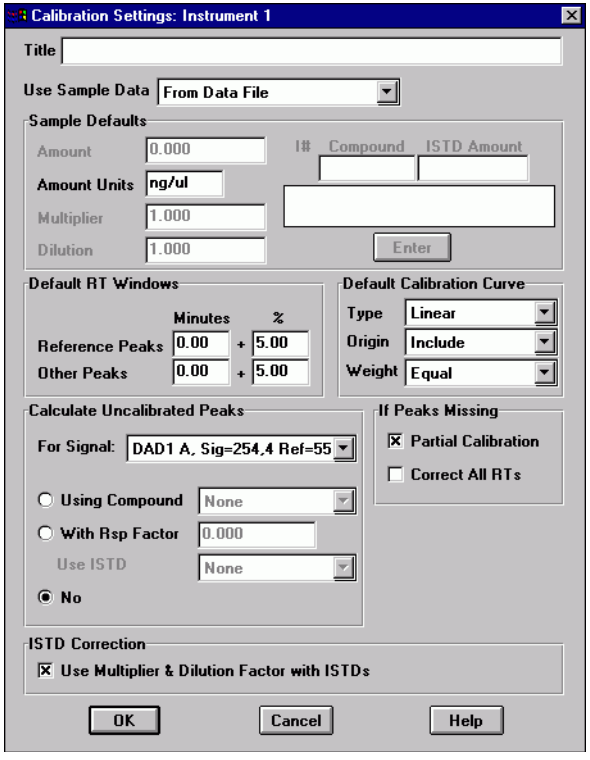

Figure 11 The Calibration Settings dialog box

2 In the **Default Calibration Curve** group, set the **Type** to **Power**.

Note the change to the calibration curve.

3 Click **OK** to close the dialog box.

4 Requantify the unknown and note the change in the results:

Report > Print Report

Recalibrating a Level

1 Load the signal DAD1 A of the data file 005-0103.D from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

2 Display the **Recalibrate** dialog box (see Figure 12):

Calibration > Recalibrate

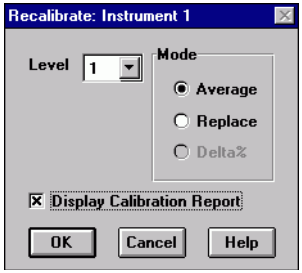

Figure 12 The Recalibrate dialog box

3 In the **Recalibrate** dialog box, set the **Level** to `1` and the **Mode** to **Average**.

4 Click **OK** to close the dialog box

5 Select **Display Calibration Report.**

6 When the report is displayed, note the changes, then close the report and accept the recalibration.

7 Load the signal DAD1 A of the data file 005-0102.D from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

8 Print a quantitative report to the screen and the printer:

Report > Print Report

Setting Up a Single-Level ISTD Calibration

This exercise uses the same data files as for the ESTD calibration, but identifies the second peak as an internal standard.

1 Load the default method, *def_lc.m*:

File > Load > Method

2 Load the signal *DAD1 A* of the data file *005-0101.D* from the **DEMO** folder.

See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

3 Display the Calibrate dialog box (see Figure 9 on page 33):

Calibration > New Calibration Table

4 In the **Calibration Table** group:

a Select the **Automatic Setup** option.

b Set the **Level** to `1`.

c Set the **Default Amount** to `100.`

5 Click **OK** to close the dialog box and set up the calibration table.

6 In the **Compound** column of the calibration table, enter names for the three compounds and the internal standard (for example, `Compound 1`, `IntStd`, `Compound 2` and `Compound 3`).

7 Click in the **ISTD** column of peak 2, select the down arrow and select **Yes**, then click elsewhere in the calibration table to display the **Calibration Table** dialog box (see Figure 13).

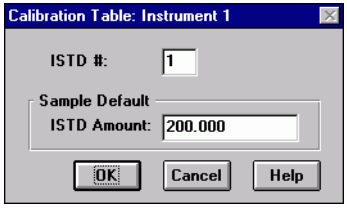

Figure 13 The Calibration Table dialog box

8 In the **Calibration Table** dialog box,

   a Set the **ISTD #** to `1`.

   b Set the **ISTD Amount** to `200`.

   c Click **OK** to close the **Calibration Table** dialog box.

9 In the **Amt** column of peak 2, set the amount of the internal standard to `200`.

10 Display the **Specify Report** dialog box:

   Report > Specify Report

11 In the **Destination** group, select **Printer** and **Screen.**

12 In the **Quantitative Results** group:

   a Click the down arrow of the **Calculate** combo box and select **ISTD** from the list.

   b Ensure that **Based On** is set to **Area**, and **Sorted By** is set to **Signal**.

13 Save the method as `testcal2.m`:

   File > Save As > Method

14 Load the signal *DAD1 A* of the data file *005-0102.D* from the **DEMO** folder.

   See step 3 of "Setting Up a Single-level ESTD Calibration" on page 31 for detailed instructions.

15 Print a quantitative report to the screen and the printer:

   Report > Print Report

5 Automating Analyses

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This exercise guides you through the process of setting up a sequence.

Before You Start

Before you start this exercise, ensure that:

- you have a number of samples that you want to run automatically,
- the samples will all run under the same chromatographic conditions (column, solvent system).

Setting Up the Sequence Parameters

1 If necessary, switch to Method and Run Control view:

View > Method and Run Control (see Figure 14)

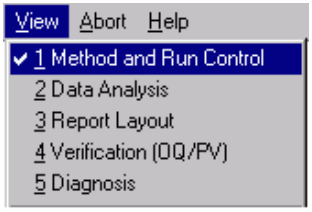

Figure 14 Switching to Method and Run Control view

2 Click in the main toolbar to switch to the Sequence panel.

3 Load the default sequence, *def_lc.s*:

File > Load > Sequence

4 Display the **Sequence Parameters** dialog box (see Figure 15 on page 44):

Sequence > Sequence Parameters

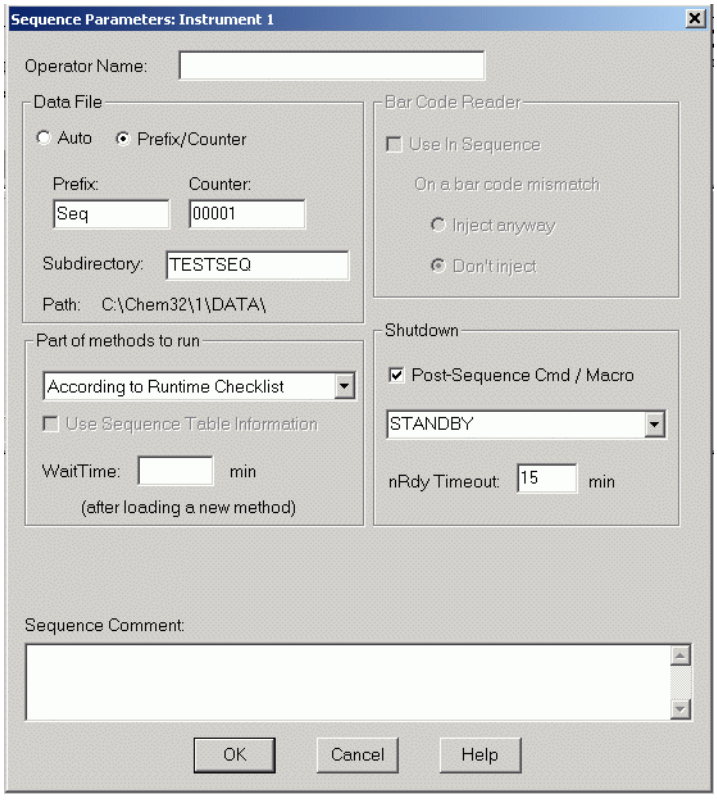

Figure 15 The Sequence Parameters dialog box

5 In the **Sequence Parameters** dialog box, enter `your name` in the **Operator Name** field.

6 In the **Data File** group:

- **a** Select **Prefix/Counter**
- **b** Enter a prefix name, for example, `Seq`
- **c** Leave the counter set to its default value (`0001`).
- **d** Enter a **Subdirectory** name, for example, `TestSeq` and click elsewhere in the dialog box. Click **OK** in the message box to create the subdirectory.

These parameters automate the data file naming of your sequence samples as Seq0001, Seq0002, . . . , Seq000n, placing them in the new subdirectory TestSeq.

e In the **Shutdown** group, select **Post-sequence Cmd/Macro**.

f Click the down arrow and select **STANDBY**.

g Set an **nRdy Timeout** to `15 minutes`.

This ensures that, in the case of an error condition, the system shuts down cleanly after 15 minutes of non-operation.

Click **OK** to close the **Sequence Parameters** dialog box.

Setting Up the Sequence Table

1 Display the **Sequence Table** (see Figure 16):

Sequence > Sequence Table

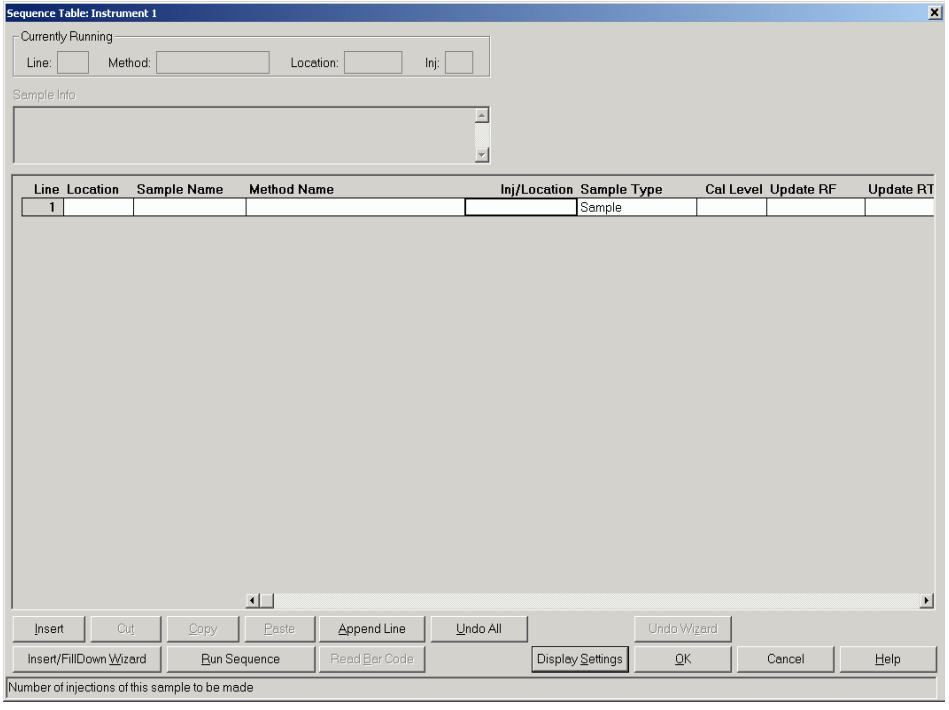

Figure 16 The Sequence Table

2 Complete the first line of the **Sequence Table** with the mandatory information:

a Enter a **Sample Location**.

b Click in the **Method** cell, display the drop-down list and select a method.

c Enter a number of injections for this location.

You can also choose to complete the other fields. Note that the horizontal scroll bar gives you access to columns at the right of the table. See the on-line help and the Understanding Your ChemStation manual for more information.

3 Click **Append Line** to add a new line to the table, and complete the line as before.

4 When you have completed a line in the **Sequence Table** for each of your samples, click **OK** to close the **Sequence Table**.

5 Save the sequence with a new name:

Sequence > Save Sequence As.

Running the Sequence

1 Ensure that your samples are in the correct positions in the autosampler tray, according to their entries in the Sequence Table.

2 Click

to start the sequence.

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In This Book

This book provides step-by-step instructions to get you started with using the Agilent ChemStation. The following tasks are covered:

- Equilibrating the System
- Setting Up a Method to Run a Checkout Sample
- Integrating the Signal
- Setting Up Calibrations
- Automating Analyses

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